J. Physiol. (1955) 128, 547–556

SUSPENDED ANIMATION IN COOLED, SUPERCOOLED AND FROZEN RATS

BY R. K. ANDJUS*

From the National Institute for Medical Research, Mill Hill, London, N.W. 7

(*Received* 3 *December* 1954)

Not long ago it was generally believed that the lowest level of hypothermia compatible with survival was around 15° C for the adult rat, as well as for the majority of non-hibernating mammals (Adolph, 1948). We have shown, however, that it is possible to reanimate the adult rat after cooling to much lower body temperatures (0–1° C) and in spite of prolonged respiratory and cardiac arrest (Andjus, 1951*a*; Andjus & Smith, 1954, 1955). Complete recovery and long-term survival in 80–100% of animals have been obtained using microwave diathermy for resuscitation (Andjus & Lovelock, 1955). When this method of reanimation which ensured a high percentage of recovery had been established, a start was made in investigating a number of related problems. These included the maximum duration of suspended animation, the effects of repeated coolings to zero, and the possibility of reanimating rats cooled to subzero temperatures. The results so far obtained along these lines will be described here.

MATERIAL AND METHODS

Animals

Rats of the Medical Research Council hooded strain were used as before. The animals ranged in weight from 160 to 210 g, except those cooled to below 0° C which were 85–105 g in body weight.

Cooling methods

Stage I (the closed vessel technique) and stage II (in crushed ice) have been described previously (Andjus & Smith, 1954, 1955). The third stage of cooling aimed at reducing the body temperature below 0° C by a modification of the method described for the hamster (Smith, Lovelock & Parkes, 1954). The temperature of the propylene glycol bath varied from −5° to −20° C in different experiments. The animals were fully immersed. The paws and the head were thickly coated with vaseline.

Colonic temperatures above zero were recorded with mercury thermometers. Subzero temperatures in the colon and subcutaneous tissue of the back were recorded from thermocouples connected to a potentiometer using a galvanometer as a null-point indicator. An error not less than ±0·3° C must be taken into account in considering the absolute values.

* Of the Faculty of Science, University of Belgrade.

Technique of reanimation

Unless otherwise stated, methods already described were used for reanimation by microwave diathermy (Andjus & Lovelock, 1955) and for rewarming in the final stages (Andjus & Smith, 1955).

RESULTS

Time limits

Six groups of ten rats each were cooled to colonic temperatures of 0–1° C. Each group was, however, kept for a different length of time in that range before reanimation was attempted.

It has been established that soon after the colonic temperature falls below 15° C, cessation of breathing followed by cardiac arrest occurs and the possibility of spontaneous recovery in a warm atmosphere is lost. During reanimation, on the other hand, the heart beat is re-established after only a few minutes of heating. Therefore, the length of time spent below 15° C prior to the application of heat roughly corresponds to the period of 'suspended animation'. This was the time interval taken into account for comparison between the groups. It will be referred to in future as the period of suspended animation.

TABLE 1. The survival of rats after varying periods of suspended animation. Each group consisted of 10 rats.

Group no.	Duration of suspended animation (min)	No. of rats: Recovered completely	Dead within 10 days	Dead within 24 hr	Failed to revive
1	60–70	10	0	0	0
2	70–80	6	2	1	1
3	80–90	4	2	3	1
4	90–100	3	0	2	5
5	100–110	1	0	4	5
6	110–120	0	0	1	9

TABLE 2. The terminal blood-sugar concentration in rats dying shortly after reanimation

Interval between reanimation and death (hr)	No. of rats	Terminal blood-sugar concentration (mg %)
0	3	202
1	4	126
$1\frac{1}{2}$	2	108
$2\frac{1}{4}$	3	112
$3\frac{1}{2}$	2	70

Table 1 shows the final results of these experiments. It will be seen that ten out of ten animals cooled to zero recovered completely when the period of suspended animation was 60–70 min. The longer the period of suspended animation the smaller the proportion of animals revived, and only one out of ten recovered when the time interval was extended to 100–110 min. In the group kept in suspended animation for 110–120 min the one animal which was reanimated died during final rewarming. Delayed deaths occurred frequently in animals revived after 70–100 min suspended animation. Most of the deaths in the second group (70–80 min) occurred with a delay of several days. Symptoms accompanying this secondary death were similar to those previously described, such as impaired thermoregulation and haemorrhage in the small intestine and stomach. Blood-sugar levels at the moment of death were correlated with the duration of survival after reanimation, as shown in Table 2. When death occurred shortly after reanimation the rats were hyperglycaemic. When survival was longer they were slightly hypoglycaemic at the time of death.

Repeated cooling and reanimation

A series of rats was repeatedly cooled to 0–0·5° C. They were kept each time for 60–70 min in the range of suspended animation.

Fig. 1 A shows the growth curve of a rat cooled to zero on alternate days. Its growth is compared with that of a control animal. The rat tolerated eight coolings within 16 days. Its weight, however, fell considerably. The animal died 18 days after the last cooling without regaining its initial weight. Its body temperature was unstable all the time. The growth curve of another rat repeatedly cooled to zero is shown in Fig. 1 B. In this instance the interval between each successive cooling was longer, especially after the first cooling when the animal was allowed to recover its initial weight over a period of 15 days. During the sixth cooling the rat was kept in suspended animation for a longer period (80 min) and the seventh cooling was carried out only 6 days later. This animal tolerated ten coolings to zero within 43 days and recovered completely. Its growth was arrested during the whole period of repeated cooling, but was resumed afterwards.

Subsequently rats were repeatedly cooled to zero, but each time the animal was allowed to regain its initial weight before it was cooled again. It was noticed that during successive experiments the time necessary to regain initial weight after each cooling tended to decrease. For example, one rat needed 11 days to regain its initial weight after the first cooling, 6 days after the second, and 1–3 days after the third to eighth cooling. The means taken from the results obtained with a group of seventeen animals show the same tendency (Table 3).

Further improvements in the recovery after reanimation were noted in repeatedly cooled rats. For a few hours after the first reanimation and artificial rewarming to 37° C the rat is not able to maintain its normal body temperature in a cold environment. When left in a refrigerator at 0 to +3° C the reanimated rat steadily cools down. By contrast, a number of rats reanimated for the sixth to eighth time were perfectly capable of maintaining their normal body temperature in the refrigerator (Table 4 and Fig. 2).

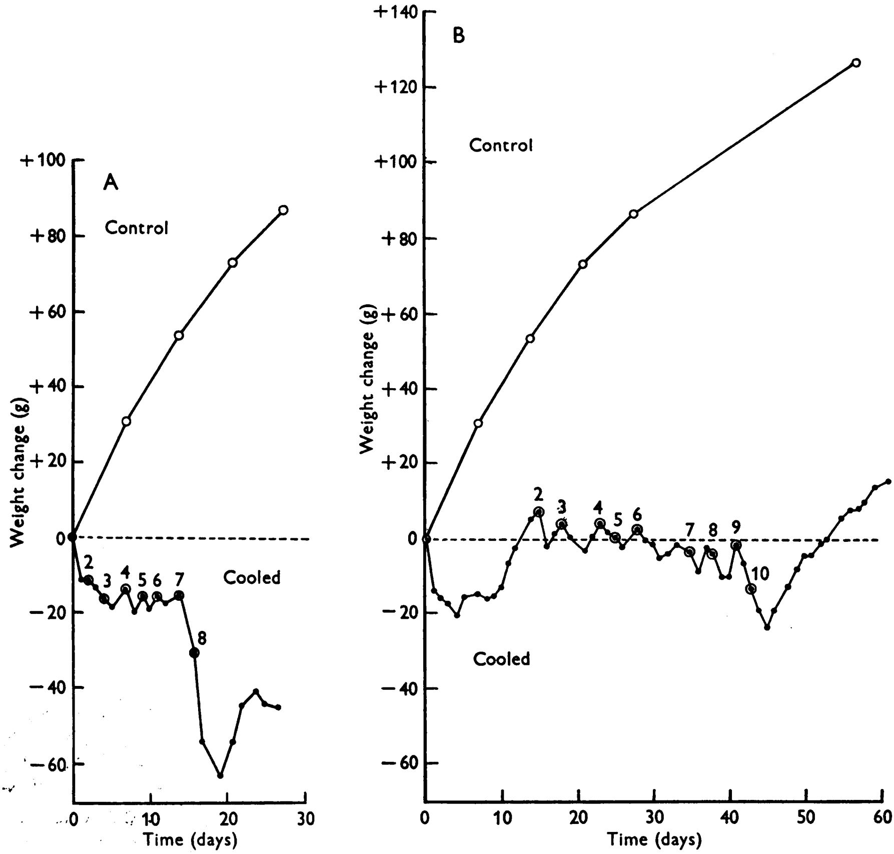

Fig. 1. The alteration in weight of control rats and of rats repeatedly cooled. The circles with accompanying numbers refer to the days on which cooling was performed. A. The experimental rat was cooled 8 times on alternate days; B, The experimental rat was cooled 10 times during the space of 43 days.

TABLE 3. The effect of repeated cooling on recovery of weight

No. of times cooled	No. of animals	Average time to regain initial weight (days)
1	17	6·65
2	15	3·65
3	16	3·15
4	10	2·85
5–10	14	1·8

It was also noted that rats reanimated for the first time, and having just resumed their heart beat and respiration, with a body temperature of 15° C (see Andjus & Smith, 1955) were not capable of spontaneous rewarming to 37° C when left at room temperature (21–23° C), and died after a few hours

TABLE 4. Effect of exposure to an ambient temperature of 0° to +3° C on the body temperature of rats 1 hr after revival (see text)

No. of rat	Weight (g)	No. of times successively revived	Time in refrigerator at 0–3° C (min)	Body temperature (° C) Initial	Final
R. 1	202	1	110	36·8	27·3
R. 2	203	1	90	37·7	29·8
R. 3	198	1	90	38·2	31·2
R. 4	127	1	90	38·2	30·3
R. 5	207	1	120	37·9	32·2
R. 6	201	6	130	38·2	36·8
R. 7	208	7	120	37·8	36·9
R. 8	200	6	120	38·2	37·7
R. 9	175	7	120	38·1	37·3
R. 10	167	5	120	38·2	36·6

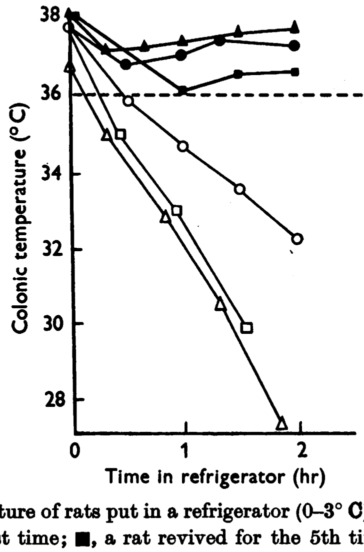

Fig. 2. The colonic temperature of rats put in a refrigerator (0–3° C) 1 hr after revival. ○, △, □, rats revived for the first time; ■, a rat revived for the 5th time; ▲, a rat revived for the 6th time; ● a rat revived for the 7th time.

if the rewarming was not completed artificially. By contrast, a number of rats reanimated for the fifth to eighth time spontaneously regained their normal body temperature when left on the bench with colonic temperatures of 15° C (Table 5 and Fig. 3).

Finally, an attempt was made to find out whether a rat after being cooled several times to zero is capable of tolerating a longer period of suspended animation. One rat cooled for the third time to zero, and another cooled for the sixth time, were kept in suspended animation for 120 min. They were both reanimated, recovered completely and survived for many months. Ten controls, cooled for the first time, did not even recover spontaneous breathing.

TABLE 5. The final body temperature of recently reanimated rats kept at room temperature (21–23° C) for 4 hr. In each experiment the initial colonic temperature was 15° C.

No. of rat	Weight (g)	No. of times successively revived	Final body temperature reached (°C)
G. 1	193	1	23·4
G. 2	175	1	21·3
G. 3	213	1	25·4
G. 4	216	1	25·8
G. 5	195	1	23·2
G. 6	199	5	31·4
G. 7	188	5	37·9
G. 8	176	5	37·0
G. 9	178	8	37·2
G. 10	218	8	38·0

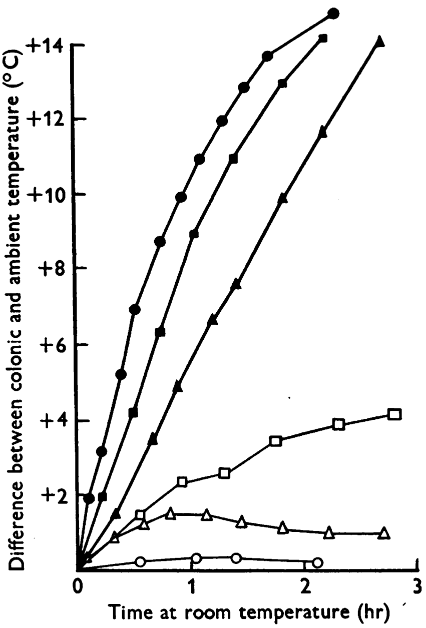

Fig. 3. The rise above room temperature of the colonic temperature of recently reanimated rats. ○, △, □, rats reanimated for the first time; ▲, a rat reanimated for the 5th time; ●, ■, rats reanimated for the 8th time.

These results were taken as an indication of some kind of adaptation produced by repeated cooling and reanimation. The basal metabolic rate was, therefore, measured in order to find out whether repeated cooling produces some of the characteristic reactions elicited by adaptation to cold environment. It is well established that prolonged exposure to cold environment results in an increased B.M.R. The figures obtained in measuring the B.M.R. of rats kept for a month in an incubator (about 30° C), and of rats exposed to a temperature of 0–10° C during the winter months, agreed with those published by Gelineo (1933). The B.M.R. of rats after several coolings to and revival from zero was not in the range characteristic for cold adaptation. It was closer to that of heat-adapted animals (Table 6).

TABLE 6. The B.M.R. of heat-adapted and cold-adapted rats and of rats 2–6 days after the last of a series of reanimations from 0 to +0·5° C

Group	No. of rat	Weight (g)	B.M.R. (cal/m²/24 hr)
Heat-adapted	Rh. 1	186	654
	Rh. 2	210	743
	Rh. 3	250	732
	Data from Gelineo (1933)	—	628
Cold-adapted	Rc. 1	175	983
	Rc. 2	204	822
	Data from Gelineo (1933)	—	930
Repeatedly cooled and revived (number of coolings shown in brackets)	G. 63 (7)	208	679
	G. 114 (7)	174	701
	G. 114 (9)	198	683
	G. 106 (5)	199	776
	G. 162 (5)	168	762

TABLE 7. The weights of the adrenal glands of rats after one or more successive coolings

No. of times cooled	No. of rats	Adrenal weight (mg/100 g body wt.)	
		Average	Range
1	13	15·4	12·0–19·5
2	7	22·4	15·6–26·8
3	6	24·8	17·2–26·4
5	7	25·2	20·4–32·0
6–9	6	23·6	21·2–28·4

Autopsies were performed on the rats which failed to revive after one or more coolings and the adrenals were weighed. Those which had been cooled repeatedly had larger adrenals than rats of the same weight range which had been cooled only once (Table 7).

Subzero range

We have previously reported that partial recovery of rats can be obtained even after their colonic temperature has been reduced slightly below zero, and after a layer of peripheral tissues has been frozen solid (Andjus, 1951*b*). In these early experiments freezing was achieved simply by covering the rat, in the third stage of cooling, with a mixture of crushed ice and salt. The technique used for reanimation was a less efficient one (heating the praecordium by applying a hot spatula to the chest wall).

In present experiments, however, the new technique of reanimation by microwave diathermy was used. The cooling procedure in stage III was basically the same as that described for the supercooling and freezing of hamsters (Smith *et al.* 1954).

The main object was to find out if substantial supercooling of the rat could be achieved in the same way as in the hamster, with subsequent complete recovery.

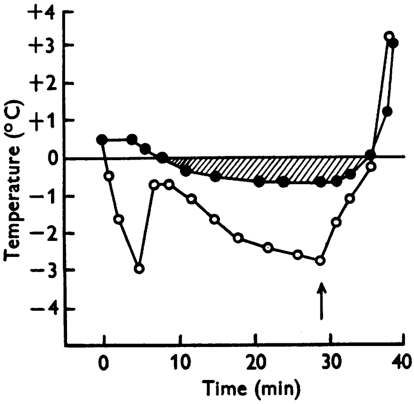

Fig. 4

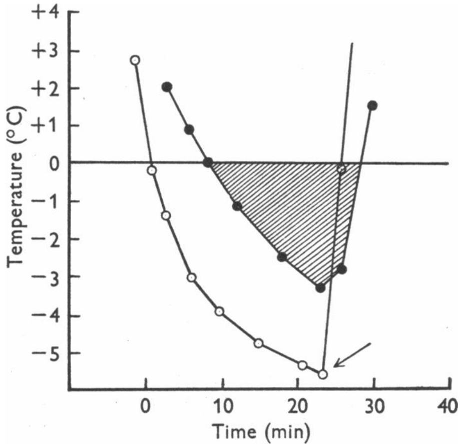

Fig. 5

Fig. 4. Cooling curves obtained from a rat immersed in a bath at −7·5° C. Arrow indicates start of rewarming. ○—○, subcutaneous temperature; ●—● colonic temperature.

Fig. 5. Cooling curves obtained from a rat immersed in a bath at −6° C. Arrow indicates start of rewarming. ○—○, subcutaneous temperature; ●—● colonic temperature.

Experiments described above had shown the importance of time-relations, and the aim was to reach subzero levels within the time limits compatible with the maximum survival rate in rats cooled to zero. The whole period of suspended animation did not exceed 60–70 min. In order to obtain a rate of cooling comparable to that described for hamsters weighing about 100 g, rats of similar weight were used.

Two typical experiments are illustrated in Figs. 4 and 5.

Data in Fig. 4 were obtained from a rat undergoing crystallization, while Fig. 5 illustrates supercooling.

It can be seen in Fig. 4 that soon after reaching a few degrees below zero, a sudden rise of the subcutaneous temperature occurred spontaneously. It marks the onset of crystallization. After rising from −3° to −0·7° C, the subcutaneous temperature begins to fall again, at a slower rate. This corresponds to the cooling of the frozen tissue. The colonic temperature, however, slowly reaches −0·7° C, and then remains at the same level until rewarming. The 'freezing plateau' is very similar to that described for the hamster.

By contrast, it can be seen in Fig. 5 that in this animal both subcutaneous and colonic temperatures continued to fall below the freezing-point without interruption. Supercooling continued until the temperature reached $-5{\cdot}7^\circ$ C under the skin and $-3{\cdot}2^\circ$ C in the colon. Three rats were supercooled by immersion in a propylene glycol bath at -5° to -6° C in the third stage of cooling. Their colonic temperatures reached $-2{\cdot}1^\circ$, $-2{\cdot}9^\circ$ and $-3{\cdot}3^\circ$ C respectively. The lowest subcutaneous temperature recorded was $-5{\cdot}7^\circ$ C (see Fig. 5). The longest period spent in the subzero range was 40 min in the instance of the rat revived after supercooling to the colonic temperature of $-2{\cdot}9^\circ$ C. All three of the rats supercooled without crystallization were reanimated, recovered completely, and resumed growth.

In eight out of nine animals that underwent crystallization in a propylene glycol bath at -7° C or below, heart beat and spontaneous breathing were re-established. All died, however, during the last stage of rewarming or within the first 24 hr after reanimation.

DISCUSSION

It has been established that an adult rat can be kept alive for 10–12 hr when its body temperature is maintained at 15–18° C (Adolph, 1948). In the experiments now described the maximum survival time at a body temperature of 0–1° C was much shorter than at 15–18° C, and in fact did not exceed 2 hr. One might have expected that at the lower temperatures the survival time would be longer. It must, however, be taken into account that at the lower range respiration and circulation are arrested completely, and this may be an important limiting factor. Other deleterious factors may come into play at this temperature, e.g. damage to capillaries, disturbances of enzymic systems, physico-chemical changes in the distribution of lipids, and even perhaps alterations in cellular components of the blood stream.

Hitherto it has been the rule to compare the ability of the newborn rat to withstand arrested circulation and respiration at reduced body temperatures with the relative intolerance of the adult rat and the relative tolerance of poikilothermic animals to these conditions (Adolph, 1951; Fairfield, 1948). One hour of cardiac arrest at body temperatures in the vicinity of 0° C was reported as critical even in poikilotherms (Macko & Selivanova, 1949). In this respect there now appears to be less difference than hitherto supposed between the adult and the newborn rat, and even between the rat and the poikilotherms. According to Adolph (1948, 1950), adaptation to a cold environment did not alter the lethal limits of hypothermia in the rat. Popović (1952) showed that rats could tolerate repeated coolings on successive days to body temperatures of 15° C, but he did not report on adaptive changes. It is therefore of particular interest that rats have now been repeatedly cooled to 0° C and subjected to prolonged periods of suspended animation. Further-more, repetition of the treatment seemed to improve their capacity for recovery. The physiological explanation of these data remains to be found.

The few results obtained with the supercooled and frozen rat show that the ability to tolerate subzero temperatures is not a peculiarity of hibernating mammals.

Much further work remains to be done on the biochemical and physical changes occurring in rats exposed to subzero temperatures and in determining optimal conditions for resuscitation, by adaptation or other means.

SUMMARY

1. The maximum duration of suspended animation compatible with survival in rats cooled to body temperatures of 0 to 1° C was determined.

2. Rats were repeatedly cooled to 0° C up to 10 times. Indications of adaptive changes were observed.

3. Rats supercooled to about −3° C colonic temperature were reanimated and recovered completely.

My best thanks are due to Sir Charles Harington, F.R.S., and the Medical Research Council for enabling me to spend a year at the National Institute for Medical Research in the Division of Experimental Biology with Dr A. S. Parkes, F.R.S., and his colleagues.

Dr Audrey Smith gave invaluable help in preparing the manuscript.

The kind and expert technical assistance of Miss J. Ashworth is gratefully acknowledged.

REFERENCES

Adolph, E. F. (1948). Lethal limits of cold immersion in adult rats. *Amer. J. Physiol.* **155**, 378–387.

Adolph, E. F. (1950). Oxygen consumptions of hypothermic rats and acclimatization to cold. *Amer. J. Physiol.* **161**, 359–373.

Adolph. E. F. (1951). Responses to hypothermia in several species of infant mammals. *Amer. J. Physiol.* **166**, 75–91.

Andjus, R. (1951*a*). Sur la possibilité de ranimer le rat adulte refroidi jusqu'à proximité du point de congélation. *C.R. Acad. sci., Paris*, **232**, 1591–1593.

Andjus, R. (1951*b*). O mogućnosti oživljavanja odraslog pacova ohladjenog do blizu tačke mržnjenja. *'Glas'. Serb. Acad. Sci.* (*Belgrade*), **200**, 249–255.

Andjus, R. K. & Lovelock, J. E. (1955). Reanimation of rats from body temperatures between 0 and 1° C by microwave diathermy. *J. Physiol.* **128**, 541–546.

Andjus, R. K. & Smith, A. U. (1954). Revival of hypothermic rats after arrest of circulation and respiration. *J. Physiol.* **123**, 66–67*P*.

Andjus, R. K. & Smith, A. U. (1955). Reanimation of adult rats from body temperatures between 0 and 2° C. *J. Physiol.* **128**, 446–472.

Fairfield, J. (1948). Effects of cold on infant rats: body temperatures, oxygen consumption, electrocardiograms. *Amer. J. Physiol.* **155**, 355–365.

Gelineo, S. (1933). Prilagodjavanje termogeneze na toplotnu sredinu. Thesis, University of Belgrade.

Macko, S. N. & Selivanova, V. M. (1949). Vosstanovlenie zhiznennykh funktsii v zavisimosti ot vremeni ikh ischeznoveniya u podvergnutykh zamorazhivaniyu pozvonochnykh zhivotnykh. *C.R. Acad. Sci. U.R.S.S.* **69**, 469–472.

Popović, V. (1952). Prilozi termogenezi ohladjenog homeoterma. Thesis, University of Belgrade.

Smith, A. U., Lovelock, J. E. & Parkes, A. S. (1954). Resuscitation of hamsters after supercooling or partial crystallisation at body temperatures below 0° C. *Nature, Lond.*, **173**, 1136–1137.